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(54) Title: ASSAY TECHNIQUE			
(57) Abstract <p>An assay technique for the qualitative and/or quantitative detection of a chemical, biochemical or biological detection of a chemical, biochemical or biological species in a sample. The technique comprises (a) coating at least a predetermined part of a pre-formed surface on a substrate with a thin film of a material capable of binding the species to be assayed, the pre-formed surface being optically active with respect to radiation at least over a predetermined band of wavelengths; (b) contacting the coated surface with the sample; and (c) observing the optical properties of said pre-formed surface in order to determine a qualitative and/or quantitative change in optical properties as a result of the binding of the species onto said thin film of material. The optical properties of the pre-formed surface may be observed before and after step (b) in order to determine any change in optical properties, or they may be monitored during step (b). The pre-formed surface is preferably a grating. An article for use in the above technique is also disclosed, and comprises a substrate carrying said pre-formed surface which in turn is coated with the receptive material for the species to be assayed.</p>			

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## AMENDED CLAIMS

[received by the International Bureau on 12 July 1984 (12.07.84);  
original claims 1 to 71 have been replaced by amended claims 1 to 72]

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1. An assay technique for qualitative and/or quantitative detection of a chemical, biochemical or biological species in a sample, which comprises (a) coating at least a predetermined part of a surface having a pre-formed relief profile on a substrate with a thin film of a material capable of binding the species to be assayed, said surface part being optically active with respect to radiation at least over a predetermined band of wavelengths; (b) contacting the coated surface with the sample; and (c) observing the optical properties of said surface part in order to determine a qualitative and/or quantitative change in optical properties as a result of the binding of the species onto said thin film of material.

2. A method according to claim 1, wherein the optical properties of said surface part are observed before and after step (b) in order to determine the said change in optical properties.

3. A method according to claim 1, wherein the optical properties of said surface part are monitored during step (b) in order to determine the said change in optical properties.

4. A method according to claim 1, 2 or 3, wherein said pre-formed surface relief profile is a diffraction grating.

5. A method according to claim 1, 2 or 3, wherein said pre-formed surface relief profile comprises two or more diffraction gratings disposed mutually at an angle.

6. A method according to claim 4 or 5, wherein the or each grating is of square-wave profile.

7. A method according to claim 4 or 5, wherein the or each grating is of sinusoidal profile.

8. A method according to claim 4 or 5, wherein the or each grating is of saw-tooth profile.



1           9. A method according to claim 1, 2 or 3, wherein  
said pre-formed surface relief profile comprises a  
regular array of protuberances.

          10. A method according to claim 4, 5, 6, 7, 8 or  
5   9, wherein said thin film of material is coated onto said  
surface so as to be deposited only in the troughs of the  
grating or in the troughs between the protuberances.

          11. A method according to any one of claims 4 to  
10, wherein the or each grating or the protuberances has  
or have a depth (peak-to-trough) in the range 10 to 50  
nanometers.

          12. A method according to any one of claims 4 to  
10, wherein the or each grating or the protuberances has  
or have a depth (peak-to-trough) in the range 50 to 200  
15 nanometers.

          13. A method according to any one of claims 4 to  
10, wherein the or each grating or the protuberances has  
or have a depth (peak-to-trough) in the range 200-2000  
nanometers.

20           14. A method according to claim 12 or 13, wherein  
the pitch (period) of the or each grating or the  
periodicity of the protuberances is of the same order as  
their depth.

          15. A method according to claim 11, wherein the  
25 pitch (period) of the or each grating or the periodicity  
of the protuberances is greater than their depth.

          16. A method according to any one of claims 1 to  
10, wherein said surface is structured so that it is  
optically active with respect to radiation of wavelengths  
30 from 700 to 1500 nanometers.

          17. A method according to any one of claims 1 to  
10, wherein said surface is structured so that it is  
optically active to radiation of a wavelength in the  
range from 350 to 700 nanometers.

35           18. A method according to any preceding claim,  
wherein the substrate is formed of a plastics material.

          19. A method according to claim 18, wherein said



1 plastics material is a material which is curable by  
ultra-violet light.

20. A method according to claim 18 or 19, wherein  
said plastics material is an acrylic or a polyester.  
5 material.

21. A method according to claim 20, wherein said  
plastics material is polymethylmethacrylate.

22. A method according to any one of claims 1 to  
17, wherein the substrate is a glass coated with a  
10 synthetic polymeric material.

23. A method according to any one of claims 1 to  
17, wherein at least the active surface of the substrate  
is constituted by a metal or a metal layer.

24. A method according to any one of claims 1 to  
15 21, wherein the active surface of the substrate is  
constituted by an inorganic oxide or a layer thereof.

25. A method according to claim 23, wherein said  
metal is gold, silver, copper or aluminium.

26. A method according to claim 24, wherein said  
20 inorganic oxide is an oxide of silver, copper or  
aluminium.

27. A method according to any preceding claim,  
wherein the substrate is in strip-form.

28. A method according to claim 18, 19, 20 or 21,  
25 wherein the plastics material has a refractive index in  
the range 1.25 to 1.6.

29. A method according to claim 28, wherein the  
refractive index of said plastics material is about 1.4.

30. A method according to any preceding claim,  
30 wherein in step (c) the optical properties of said  
pre-formed surface are observed in transmission.

31. A method according to any one of claims 1 to  
29 wherein in step (c) the optical properties of said  
pre-formed surface are observed in reflection.

32. A method according to any preceding claim,  
35 wherein one zone of said surface distinct from said  
predetermined part of the surface on the substrate is



1 left free of the coating material and is not contacted,  
in step (b), by the sample.

33. A method according to any one of claims 1 to  
31, wherein one zone of said surface distinct from said  
5 predetermined part of the surface is left free of the  
coating material and the whole of said surface, including  
said one zone, is contacted, in step (b), by the sample.

34. A method according to claim 32 or 33, wherein  
a two-beam illuminating system is employed in step (c),  
10 one of said beams being directed at said one zone of said  
surface, and the other of the two beams being directed at  
said predetermined surface part.

35. A method according to any preceding claim,  
wherein in step (c) monochromatic radiation is used.

15 36. A method according to any preceding claim,  
wherein the species which is to be detected is an  
antigen.

37. A method according to claim 36, wherein the  
material capable of binding said species comprises  
20 antibodies for the antigen which is to be assayed.

38. A method according to claim 37, wherein said  
antibodies are monoclonal antibodies.

39. A method according to any one of claims 1 to  
36, wherein the species which is to be assayed is an  
25 ionic species.

40. A method according to claim 39, wherein said  
ionic species is a metal ion.

41. A method according to claim 39 or 40, wherein  
the material capable of binding the species to be assayed  
30 is a chelating enzyme or a chelating organism.

42. A method according to claim 41, wherein said  
enzyme or organism is one or more of: a polypeptide, a  
steroid, a saccharide or polysaccharide, a proteoglycan,  
a nucleotide, a nucleic acid, a protonucleic acid, a  
35 microbial cell or a yeast.

43. A method according to any preceding claim,  
wherein said thin film of material is firmly bonded to



1 said surface.

44. A method according to claim 43, wherein said thin film of material is bonded to the said surface by electrostatic or covalent bonding.

5 45. A method according to any preceding claim, wherein, in step (c), polarised light is used to observe the optical properties of <sup>said</sup> surface part.

46. A method according to claim 45, wherein the pre-formed surface relief profile is in the form of a  
10 single grating of square-wave or sinusoidal profile, and wherein the optical properties of said surface are observed, in step (c), by monitoring the angular position at which there occurs a sharp reduction (dip) in reflection as the surface is observed or scanned with  
15 radiation of a predetermined wavelength.

47. An article for use in an assay technique for qualitative and/or quantitative detection of a chemical, biochemical or biological species in a sample, which article comprises a substrate having a surface with a  
20 pre-formed relief profile which is optically active with respect to radiation at least over a predetermined band of wavelengths, and at least a predetermined part of which surface is coated with a thin film of a material capable of binding a predetermined chemical or  
25 biochemical or biological species.

48. An article as claimed in claim 47, wherein the substrate is a plastics material.

49. An article as claimed in claim 48, wherein said plastics material is a material which is curable by  
30 ultra-violet light.

50. An article as claimed in claim 48 or 49, wherein said plastics material is an acrylic or a polyester material.

51. An article as claimed in claim 50, wherein  
35 said plastics material is polymethylmethacrylate.

52. An article as claimed in claim 47, wherein the substrate is a glass coated with a synthetic



1 polymeric material.

53. An article as claimed in any one of claims 47 to 52, wherein the substrate is lamellar.

54. An article as claimed in claim 53, wherein  
5 the substrate is in strip-form.

55. An article as claimed in claim 52, 53 or 54, wherein the pre-formed surface relief profile is in the form of a single grating or of two or more gratings disposed mutually at an angle.

10 56. An article as claimed in claim 55, wherein the or each grating is of square-wave, sinusoidal or saw-tooth profile.

57. An article as claimed in claim 52, 53 or 54, wherein the pre-formed surface relief profile comprises a  
15 regular array of protuberances.

58. An article as claimed in any one of claims 47 to 57, wherein said surface is constituted by a metal or a metal layer.

59. An article as claimed in claim 58, wherein  
20 said metal is gold, silver, copper or aluminium.

60. An article as claimed in any one of claims 47 to 59, wherein said surface is constituted by an inorganic oxide.

61. An article as claimed in claim 60, wherein  
25 said oxide is an oxide of silver, copper or aluminium.

62. An article as claimed in any one of claims 47 to 61, wherein said thin film of material comprises antibodies.

63. An article as claimed in claim 62, wherein  
30 said antibodies are monoclonal antibodies.

64. An article as claimed in any one of claims 47 to 61, wherein said thin film of material comprises a chelating enzyme or a chelating organism.

65. An article as claimed in claim 48, wherein  
35 the substrate has a refractive index in the range 1.25 to 1.6.

66. An article as claimed in claim 65, wherein





1 the refractive index of the substrate is about 1.4.

67. An article as claimed in claim 48, wherein  
said surface of the substrate when viewed in  
transmission normal to the plane of the surface with  
5 monochromatic light has a transmission not exceeding 1%.

68. An article as claimed in claim 58 or 59,  
wherein said surface is constituted by a layer at least 5  
nanometers in thickness.

69. An article as claimed in any one of claims 47  
10 to 68, wherein the article includes a plurality of zones  
each of which is coated with a different receptive  
material so that the article is capable of binding a  
plurality of different species.

70. A method according to claim 1, in which said  
15 surface is washed immediately after being contacted with  
the sample and before the observations in step (c).

71. A method according to any one of claims 1 to  
46 and 70, in which said surface is covered with a layer  
of a liquid of high refractive index between steps (b)  
20 and (c).

72. An assay technique for qualitative and/or  
quantitative detection of a chemical, biochemical or  
biological species in a sample, which comprises: (i)  
contacting the surface of an article as claimed in any  
25 one of claims 47 to 69 with the sample; and (ii)  
observing the optical properties of said surface in order  
to determine a qualitative and/or a quantitative change  
in optical properties as a result of the binding of the  
chemical, biochemical or biological species onto said  
30 surface.

